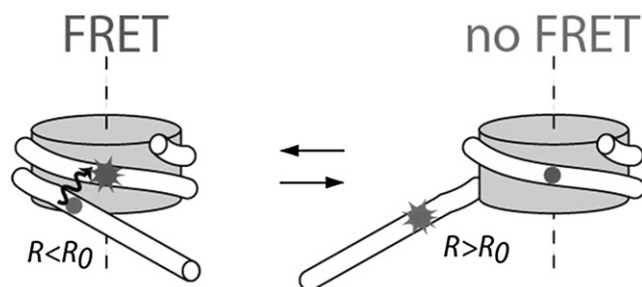


**280-Pos Board B159****Effect of Histone Acetylation on Nucleosome Dynamics Revealed by spFRET Microscopy**

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Nucleosomes form the basic unit of DNA compaction in eukaryotes. Besides condensing the DNA, nucleosomes play a crucial role in gene regulation by modulating access to the nucleosomal DNA for DNA-processing proteins. Accessibility of DNA within the nucleosome can be realized by spontaneous unwrapping (DNA breathing), and by ATP-dependent remodeling enzymes. Both mechanisms are regulated by specific post-translational modifications to the nucleosome histones. We applied single-pair FRET measurements to characterize the effect of specific histone modifications on DNA breathing dynamics in individual nucleosomes. DNA labeled with a FRET pair was used to reconstitute nucleosomes with three types of histones: native chicken erythrocyte histones, recombinant unmodified histones, and recombinant histones acetylated at H3K56. Using alternating laser excitation to select for the correct label stoichiometry, in combination with both widefield TIRF microscopy on immobilized nucleosomes and FCS on freely diffusing nucleosomes, we were able to quantify DNA breathing dynamics at timescales ranging from milliseconds to minutes. Uncovering the effect of histone modifications on the dynamic behavior of single nucleosomes provides insight in the physical mechanisms underlying gene regulation.

**281-Pos Board B160****Computer simulations of chromatin fibers**

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In the nucleus of higher organisms the DNA is organized by histone proteins into a nucleoprotein complex termed chromatin. This packing controls DNA accessibility and is therefore an important factor for the control of gene expression. We developed a new coarse-grained computer model to represent different types of chromatin fibers. Based on model structures at atomic resolution, the common two-angle nucleosome geometry was enhanced by four additional angles. The nucleosomes are modeled as spherocylinders described by an S-functions expansion and are connected by cylindrical DNA segments. Harmonic potentials for stretching, bending, and torsion represent the elastic properties of the DNA. The negative charge of the DNA is described by a Debye-Hückel-approximation. This model was used to investigate the influence of the local nucleosome geometry and the internucleosomal interaction on the chromatin fiber conformation by Monte Carlo (MC) simulations [1,2]. Three fiber types derived from experimental data of native and reconstituted chromatin were systematically analyzed. For all investigated fiber types, the simulations revealed the large impact of the nucleosome repeat length on the stability of the fiber formation. A model was proposed, in which changes of the chromatin fiber conformation induced by linker histone H1 binding as predicted from high resolution model structures are reproduced by relatively small changes of the local nucleosome geometry. Furthermore, key factors for the control of the compaction and higher order folding of the chromatin fiber were identified. We have further developed this approach and are applying it to the analysis of the conformational space of the chromatin fiber, fiber force spectroscopy experiments and atomic force microscopy imaging of chromatin fibers.

[1] Stehr, R., N. Kepper, K. Rippe, and G. Wedemann. *Biophys. J.* 95:3677 (2008).

[2] Kepper, N., D. Foethke, R. Stehr, G. Wedemann, and K. Rippe. *Biophys. J.* 95:3692 (2008).

**282-Pos Board B161****Nanomanipulation Of Single Chromatin Fiber With Magnetic Tweezers**

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The nucleosome core particle is the basic subunit of chromatin structure. It consists of ~146 DNA bp coiled leftward around an octamer that contains pairs of histones H2A, H2B, H3 and H4. Magnetic tweezers were used to study the mechanical response under torsion of single chromatin fibers. Nucleosome arrays are reconstituted on tandem repeats of 601 positioning sequences. These fibers show higher torsional plasticity than naked DNA. Such a behavior can be explained by a dynamic equilibrium between three conformations of the nucleosome, corresponding to different crossing statuses of the entry/exit DNAs: negative, null or positive. Moreover after extensive positive supercoiling these chromatin fibers display an hysteretic behavior in their mechanical response to torsion. The fibers remain more extended when they are returning to negative supercoiling values. This hysteresis is the consequence of the trapping of one positive turn per nucleosome. The results suggest a rearrangement of the nucleosome structure which can be related with the previously documented chiral transition of the tetrasome (the H3-H4 tetramer with its bound DNA). As the energy of the altered form, named reversome, is ~6 kT, these abilities to endorse torsion may be related to physiological processes such as transcription elongation since RNA polymerases generate positive supercoiling downstream. As eukaryotic chromatin contains a high proportion of linker histone we investigate the effect of H5 (avian erythrocytes variant) on the mechanical response of an array. First we produced regular fiber containing this histone in stoichiometric amount. Then we showed that even if these kind of fiber are more condensed, it still displays a high torsional plasticity and the ability to form a reversome fiber.

**283-Pos Board B162****Rapid-Quench Mixing and Use of Fast Footprinting to Characterize DNA Opening in the Late Steps of Open Complex Formation at  $\lambda$ PR by E. coli RNA Polymerase**

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The precise mechanism by which multi-subunit, DNA-dependent RNA polymerases (RNAP) recognize promoter DNA and form stable, transcriptionally-competent open complexes (RPO) remains elusive. Chemical and enzymatic footprints of early intermediates and RPO provide detailed pictures of the DNA in these initial and final complexes in transcription initiation. We have recently discovered that the start site for transcription (+1) remains double-stranded when it is first loaded into the active site channel of *E. coli* RNAP at the  $\lambda$  phase P<sub>R</sub> promoter (Davis et al. PNAS, 2007). These experiments reveal unambiguously that opening of the transcription bubble occurs subsequently in the "jaws" of RNAP as RNAP actively destabilizes the duplex. To probe whether opening of ~14 bp of DNA occurs in stages or is "all or none" we are using the perturbants urea and KCl to destabilize RPO and populate late transcription initiation intermediates. Analysis of the dissociation kinetics over a range of urea and KCl concentrations provides evidence for two transient intermediates in the DNA opening process. Using KMnO<sub>4</sub> footprinting we are probing the extent of DNA opening in these intermediates under these conditions. Results from these novel rapid (millisecond) footprinting experiments and the implications for the mechanism of promoter opening will be presented.

**284-Pos Board B163****Stress, Scrunching And Tethering: The Roles Of The Connecting Template Strand In Initiation By T7 RNA Polymerase**

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T7 RNA polymerase is the most well characterized single subunit RNA polymerase. It possesses high activity in vitro and shows high specificity for its DNA promoter. It is also functionally similar to the multi subunit RNA polymerases, making it an ideal model to study the mechanistic aspects of RNA polymerase during its transition through the three phases of transcription; initiation, elongation and termination.

Like all DNA-dependent RNA polymerases, T7 RNA polymerase carries out de novo synthesis of RNA from a double stranded template. The enzyme melts open the duplex, initiates synthesis of a dinucleotide RNA, and moves along the template DNA extending and ultimately displacing the RNA transcript. In the initially transcribing phase, the bubble expands as the RNA:DNA hybrid grows. This phase is associated with large structural rearrangements in the complex